

**REMARKS**

The Examiner is respectfully requested to enter the claim amendments herein presented prior to examination of this application on the merits. The claim amendments are proper, well within the scope and disclosure of the present invention, and do not include any new matter. Support for the breadth of the claim amendments is found in the originally filed specification, claims and drawings. In particular, these claims substantially track the identical claims allowed by the Examiner and that eventually issued as U.S. Patent No. 6,455,304. Furthermore, claims having a scope substantially commiserate as the presently amended claims were discussed with the Examiner by the Applicants' undersigned attorney on May 8, 2003 as well as in August, 2002. As such, the Examiner should be familiar with this claim language and scope of the claims and the Examiner is requested to pass these currently amended claims to an expedient issuance.

Applicants will be submitting an Information Disclosure Statement via hand-delivery to the Examiner shortly. The Examiner is directed in the interim, however, to the parent applications and co-pending applications (e.g. U.S. Serial No. 10/011,771 and U.S. Serial No. 10/011,768, in which substantial IDS' have been filed) of the present application as well as to the other related issued and pending

applications to a Group A *Streptococcal* hyaluronan synthase (e.g. U.S. Patent No. 6,455,304) for the majority of the references that will be cited by Applicants in the forthcoming Information Disclosure Statement.

In an effort to speed along the prosecution of this case, the Applicants hereinafter refer to the most pertinent references and particularly point out the deficiencies of these references.

1. WO94/00463 (MURST) January 6, 1994

While this patent application ***appears*** to claim an isolated DNA fragment comprising a nucleotide sequence encoding hyaluronic acid synthase from *S. equisimilis* D181 ("seHAS"), in reality it discloses a putative 56-kDa helper protein for the seHAS and not the seHAS enzyme itself. In short, the listed inventors found what they thought to be seHAS but was actually a different enzyme altogether. The listed inventors of the WO94/00463 application as well as other researchers in the field have published numerous articles detailing the incorrect or misidentification of the 56-kDa enzyme as the hyaluronan synthase from *S. equisimilis*.

See e.g.:

- Weigel et al., J Biol Chem 1997 Dec 19;272(51):32539-46,  
(Abstract quoted below):

We previously reported the first cloning of a functional glycosaminoglycan synthase, the hyaluronan synthase (HAS) from Group A *Streptococcus pyogenes* (spHAS) (DeAngelis, P. L., Papaconstantinou, J., and Weigel, P. H. (1993) *J. Biol. Chem.* 268, 19181-19184). Group A spHAS was unrelated to a ***putative Group C HA synthase reported by others (Lansing, M., Lellig, S., Mausolf, A., Martini, I., Crescenzi, F., Oregon, M., and Prehm, P. (1993) Biochem. J. 289, 179-184)***. Here we report the isolation of a bona fide HA synthase gene from a highly encapsulated strain of Group C *Streptococcus equisimilis*. The encoded protein, designated seHAS, is 417 amino acids long (calculated molecular weight, 47,778; calculated pI, 9.1) and is the smallest member of the HAS family identified thus far. The enzyme migrates anomalously fast in SDS-polyacrylamide gel electrophoresis (approximately 42,000 Da). The seHAS protein shows no similarity (<2% identity) to the previously reported Group C gene, which is not an HA synthase.

-- Prehm, et al., *J Biol Chem* 1998 Sep 11;273(37):23668-73, (Abstract quoted below)

***A 56-kDa protein had been isolated and cloned from protoplast membranes of group C streptococci that had erroneously been identified as hyaluronan synthase.*** The function of this protein was reexamined. When streptococcal membranes were separated on an SDS-polyacrylamide gel and renatured, a 56-kDa protein was detected that had kinase activity for a casein substrate. When this recombinant protein was expressed in *Escherichia coli* and incubated in the presence of [32P]ATP, it was responsible for phosphorylation of two proteins with 30 and 56 kDa that were not present in the control lysate. The 56-kDa protein was specifically

phosphorylated in an immunoprecipitate of a detergent extract of the recombinant *E. coli* lysate with antibodies against the 56-kDa protein, indicating that it was autophosphorylated. The *E. coli* lysate containing the recombinant protein could bind hyaluronan, and hyaluronan binding was abolished by the addition of ATP. Kinetic analysis of hyaluronan synthesis and release from isolated protoplast membranes indicated that phosphorylation by ATP stimulated hyaluronan release and synthesis. Incubation of membranes with antibodies to the 56-kDa protein increased hyaluronan release. The addition of [32P]ATP to intact streptococci led to rapid phosphorylation of two proteins, 56 and 75 kDa each at threonine residues. This phosphorylation was neither observed with [32P]phosphate nor in the presence of trypsin, indicating that the kinase was localized extracellularly. The addition of ATP to growing group C streptococci led to increased hyaluronan synthesis and release. However marked differences were found between group A and group C streptococci. Antibodies against the 56-kDa protein from group C streptococci did not recognize proteins from group A strains, and a homologous DNA sequence could not be detected by polymerase chain reaction or Southern blotting. In addition, Group A streptococci did not retain a large hyaluronan capsule like group C strains. These results indicated that the 56-kDa protein is an ectoprotein kinase specific for group C streptococci that regulates hyaluronan capsule shedding by phosphorylation.

-- Prehm S, Nickel V, Prehm P., Protein Expr Purif 1996

Jun;7(4):343-6, (Introduction quoted below):

The hyaluronate capsule is a virulence factor for the Streptococci (1). Hyaluronate is synthesized at the protoplast membrane (2).

A 42-kDa protein was identified as the synthase by transposon mutagenesis (5,6). A 56-kDa protein was previously thought to be the synthase, because it bound the precursor nucleotide sugars UDP-GlcA and UDP-GlcNAc (4,7).

#### REFERENCES

4. Prehm, P., and Mausolf, A. (1986) Biochem. J. 235, 887-889.
5. DeAngelis, P. L., Papaconstantinou, J., and Weigel, P. H. (1993) J. Biol. Chem. 268, 19181-19184.
6. Dougherty, B. A., and van de Rijn, I. (1993) J. Biol. Chem. 268, 7118-7124.
7. Lansing, M., Lellig, S., Mausolf, A., Martini, I., Crescenzi, F., O'Regan, M., and Prehm, P. (1993) Biochem. J. 289, 179-184.

Thus, the WO94/00463 patent application is an improper prior art reference for the present application because it does not teach, disclose, describe, or enable a hyaluronan synthase from *S. equisimilis*, i.e. the invention presently disclosed and defined by the amended claims.

2. DeAngelis et al., J Biol. Chem., 1993, 268(26): 19181-19184

This reference discloses the hyaluronan synthase gene from *S. pyogenes* ("spHAS") which is only 70% identical to the presently claimed seHAS. Furthermore, the spHAS does not cross-hybridize to the seHAS gene under any conditions (see e.g. pages 29-30 of the specification). Thus, the DeAngelis et al. 1993 reference does not defeat the novelty of the Applicants' claims – as it does not disclose or

suggest a gene encoding a hyaluronan synthase as defined in the amended claims.

3. Dougherty et al., J. Biol. Chem. 1994, 269(1): 169-175

As with the DeAngelis et al. 1993 reference addressed hereinabove, this reference discloses the hyaluronan synthase gene from *S. pyogenes* ("spHAS") which is only 70% identical to the presently claimed seHAS. Furthermore, the spHAS does not cross-hybridize to the seHAS gene under any conditions (see e.g. pages 29-30 of the specification). Thus, this reference does not defeat the novelty of the Applicants' claims – as it does not disclose or suggest a gene encoding a hyaluronan synthase as defined in the amended claims.

4. WO91/03559 (University of Texas)

As with the WO94/00463 MURST reference, while this patent application **appears** to claim an isolated DNA fragment comprising a nucleotide sequence encoding hyaluronic acid synthase from *S. equisimilis* D181 ("seHAS"), in reality it discloses a **histidyl tRNA synthetase**. In short, the listed inventors found what they thought to be the seHAS gene but was actually a different class and enzyme altogether.

The listed inventors of the WO91/03559 application (one of which is a co-inventor of the present application) as well as other

researchers in the field have published several articles detailing the identification of the enzyme in the WO91/03559 as the histidyl tRNA synthetase. Indeed, the U.S. corresponding issued patent to the WO91/03559 reference was recently reissued as RE 37,336 in order to correct the misidentification and patenting of the histidyl tRNA synthetase as the seHAS. In short, the inventors made a mistake as to the identification of the enzyme disclosed in the WO91/03559 application and this mistake was not discovered until after the U.S. patent issued and the WO91/03559 application was published.

See e.g.:

- Weigel et al., The histidyl-tRNA synthetase from *Streptococcus equisimilis*: overexpression in *Escherichia coli*, purification, and characterization, *Prep Biochem* 1993 Nov;23(4):449-72.
- Weigel et al., Molecular cloning, sequence, structural analysis and expression of the histidyl-tRNA synthetase gene from *Streptococcus equisimilis*, *Nucleic Acids Res* 1993 Feb 11;21(3):615-20.

In particular, note that in the Weigel et al. *Nucleic Acids Res* 1993 Feb 11;21(3):615-20 reference (which identifies and characterizes the histidyl tRNA synthetase), under the Results section on p. 616 the authors state that:

"The cloning of the *hisS* gene utilized the shuttle vector pSA3 (14), which can replicate in both *E. coli* and *Streptococcus*. An EcoRI library was constructed from *S. equisimilis* D181 genomic DNA. ... This screening strategy was devised for the isolation of a different *Streptococcus* gene, a polysaccharide synthase. "

The referenced pSA3 shuttle vector in the Weigel et al. 1993 article and the screening strategy is one in the same as the screening strategy used in the WO91/03559 application. (See e.g. Fig. 1 and specification).

Thus, while the WO91/03559 application appears to disclose the seHAS gene it actually discloses the histidyl tRNA synthetase enzyme. Thus, the WO91/03559 reference does not disclose or suggest a gene encoding a hyaluronan synthase as defined in the attached claims.

5. Semino et al., Proc. Natl. Acad. Sci. USA, 1996, 93: 4548-4553

This reference discloses a gene encoding an enzyme that was thought to catalyze synthesis of chitin oligosaccharides; it does not disclose or suggest a gene encoding a hyaluronan synthase as defined in the attached claims. Although Fig. 1 includes a portion of the HasA gene for comparison with the chitin synthase, the HasA nomenclature refers to the spHAS gene. As with the DeAngelis et al. 1993 reference addressed hereinabove, this reference discloses a portion of the hyaluronan synthase gene from *S. pyogenes* ("spHAS") which is only 70% identical to the presently claimed seHAS. Furthermore, the

spHAS gene does not cross-hybridize to the seHAS gene under any conditions (see e.g. pages 29-30 of the specification). Thus, this reference does not defeat the novelty of the Applicants' claims - as it does not disclose or suggest a gene encoding a hyaluronan synthase as defined in the amended claims.

6. WO97/20061 (Scripps)

This reference discloses the use of spHAS in an improved *in vitro* synthesis wherein released UDP is recaptured and reused during hyaluronic acid polymer synthesis. This reference discloses an *in vitro* system wherein crude membrane extracts containing unpurified native (not recombinant) spHAS catalyze the polymerization of hyaluronic acid. It does not disclose a recombinant system for producing hyaluronic acid nor does it describe or disclose a gene encoding a hyaluronan synthase as defined in the amended claims. The only novelty of any disclosed subject matter of this application is UDP-sugar regeneration, not hyaluronic acid production, due to the fact that many groups have previously used native crude hyaluronic acid synthase *in vitro* to polymerize an hyaluronic acid polymer.

As with the DeAngelis et al. 1993 reference addressed hereinabove, this reference discloses the use of a crude membrane extract containing the hyaluronan synthase gene from *S. pyogenes* ("spHAS") which is only 70% identical to the presently claimed seHAS.

Furthermore, the spHAS gene does not cross-hybridize to the seHAS gene under any conditions (see e.g. pages 29-30 of the specification). Thus, this reference does not defeat the novelty of the Applicants' claims – as it does not disclose or suggest a gene encoding a hyaluronan synthase as defined in the amended claims.

7. WO98/00551 (Mayo)

This reference discloses murine hyaluronic acid synthases, which are 21% identical and 28% identical to HasA (i.e. spHAS; see page 3, lines 10-12 of WO98/00551). With respect to the seHAS gene, the murine hyaluronic acid synthases disclosed by WO98/00551 are only approximately 10% identical to seHAS (see FIG. 3 of the present specification). Thus, this reference does not defeat the novelty of the Applicants' claims – as it does not disclose or suggest a gene encoding a hyaluronan synthase as defined in the amended claims.

8. 5,015,577

As with the WO94/00463 MURST reference, while this patent **appears** to claim an isolated DNA fragment comprising a nucleotide sequence encoding hyaluronic acid synthase from *S. equisimilis* D181 ("seHAS"), in reality it discloses a **histidyl tRNA synthetase**. In short, the listed inventors found what they thought to be the seHAS gene but was actually a different class and enzyme altogether.

The listed inventors of the 5,015,577 application (one of which is a co-inventor of the present application) as well as other researchers in the field have published several articles detailing the identification of the enzyme in the 5,015,577 as the histidyl tRNA synthetase. Indeed, the U.S. issued patent was recently reissued as RE 37,336 in order to correct the misidentification and patenting of the histidyl tRNA synthetase as the seHAS. In short, the inventors made a mistake as to the identification of the enzyme disclosed in the 5,015,577 application and this mistake was not discovered until after the U.S. patent issued.

See e.g.:

- Weigel et al., The histidyl-tRNA synthetase from *Streptococcus equisimilis*: overexpression in *Escherichia coli*, purification, and characterization, *Prep Biochem* 1993 Nov;23(4):449-72.
- Weigel et al., Molecular cloning, sequence, structural analysis and expression of the histidyl-tRNA synthetase gene from *Streptococcus equisimilis*, *Nucleic Acids Res* 1993 Feb 11;21(3):615-20.

In particular, note that in the Weigel et al. *Nucleic Acids Res* 1993 Feb 11;21(3):615-20 reference (which identifies and

characterizes the histidyl tRNA synthetase), under the Results section on p. 616 the authors state that:

"The cloning of the *hisS* gene utilized the shuttle vector pSA3 (14), which can replicate in both *E. coli* and *Streptococcus*. An EcoRI library was constructed from *S. equisimilis* D181 genomic DNA. ... This screening strategy was devised for the isolation of a different *Streptococcus* gene, a polysaccharide synthase. "

The referenced pSA3 shuttle vector in the Weigel et al. 1993 article and the screening strategy is one in the same as the screening strategy used in the 5,015,577 patent. (See e.g. Fig. 1 and specification).

Thus, while the 5,015,577 patent appears to disclose the seHAS gene it actually discloses the histidyl tRNA synthetase enzyme. Thus, the 5,015,577 patent does not disclose or suggest a gene encoding a hyaluronan synthase as defined in the attached claims.

9. Lansing M. et al. (1993) Biochem J. Vol 289: 179-184

As with the WO94/00463 (MURST) reference discussed in (a) hereinabove, while this reference appears to disclose the DNA and amino acid sequence for the hyaluronan synthase gene from *Streptococcus equisimilis*, in fact it does not disclose such a DNA or amino acid sequence. The authors of this reference misidentified an ectoprotein kinase of group C streptococci as the hyaluronan synthase

from *S. equisimilis*. As evidence of such misidentification, the Examiner is directed to the following references:

- (i) Weigel et al., J Biol Chem 1997 Dec 19;272(51):32539-46

**Abstract:** We previously reported the first cloning of a functional glycosaminoglycan synthase, the hyaluronan synthase (HAS) from Group A *Streptococcus pyogenes* (spHAS) (DeAngelis, P. L., Papaconstantinou, J., and Weigel, P. H. (1993) J. Biol. Chem. 268, 19181-19184). Group A spHAS was unrelated to a putative Group C HA synthase reported by others (Lansing, M., Lellig, S., Mausolf, A., Martini, I., Crescenzi, F., Oregon, M., and Prehm, P. (1993) Biochem. J. 289, 179-184). Here we report the isolation of a bona fide HA synthase gene from a highly encapsulated strain of Group C *Streptococcus equisimilis*. The encoded protein, designated seHAS, is 417 amino acids long (calculated molecular weight, 47,778; calculated pI, 9.1) and is the smallest member of the HAS family identified thus far. The enzyme migrates anomalously fast in SDS-polyacrylamide gel electrophoresis (approximately 42,000 Da). The seHAS protein shows no similarity (<2% identity) to the previously reported Group C gene, which is not an HA synthase. The seHAS and spHAS protein and coding sequences are 72 and 70% identical, respectively. seHAS is also similar to eukaryotic HAS1 (approximately 31% identical), HAS2 (approximately 28% identical), and HAS3 (28% identical). The deduced protein sequence of seHAS was confirmed by reactivity with a synthetic peptide antibody. Recombinant seHAS expressed in *Escherichia coli* was recovered in membranes as a major protein (approximately 10% of the total protein) and synthesized very large HA ( $M_r > 7 \times 10^6$ ) in the presence of UDP-GlcNAc and UDP-GlcA. The product contained equimolar amounts of both sugars

and was degraded by the specific *Streptomyces* hyaluronidase. Comparison of the two recombinant streptococcal enzymes in isolated membranes showed that seHAS and spHAS are essentially identical in the steady-state size distribution of HA chains they synthesize, but seHAS has an intrinsic 2-fold faster rate of chain elongation ( $V_{max}$ ) than spHAS. seHAS is the most active HA synthase identified thus far; it polymerizes HA at an average rate of 160 monosaccharides/s. The two bacterial HA synthase genes may have arisen from a common ancient gene shared with the early evolving vertebrates.

- (ii) Lansing et al., J Biol Chem 1998 Sep 11;273(37):23668-73

**Abstract:** A 56-kDa protein had been isolated and cloned from protoplast membranes of group C streptococci that had erroneously been identified as hyaluronan synthase. The function of this protein was reexamined. When streptococcal membranes were separated on an SDS-polyacrylamide gel and renatured, a 56-kDa protein was detected that had kinase activity for a casein substrate. When this recombinant protein was expressed in *Escherichia coli* and incubated in the presence of [ $^{32}$ P]ATP, it was responsible for phosphorylation of two proteins with 30 and 56 kDa that were not present in the control lysate. The 56-kDa protein was specifically phosphorylated in an immunoprecipitate of a detergent extract of the recombinant *E. coli* lysate with antibodies against the 56-kDa protein, indicating that it was autophosphorylated. The *E. coli* lysate containing the recombinant protein could bind hyaluronan, and hyaluronan binding was abolished by the addition of ATP. Kinetic analysis of hyaluronan synthesis and release from isolated protoplast membranes indicated that phosphorylation by ATP stimulated hyaluronan release and synthesis. Incubation

of membranes with antibodies to the 56-kDa protein increased hyaluronan release. The addition of [32P]ATP to intact streptococci led to rapid phosphorylation of two proteins, 56 and 75 kDa each at threonine residues. This phosphorylation was neither observed with [32P]phosphate nor in the presence of trypsin, indicating that the kinase was localized extracellularly. The addition of ATP to growing group C streptococci led to increased hyaluronan synthesis and release. However marked differences were found between group A and group C streptococci. Antibodies against the 56-kDa protein from group C streptococci did not recognize proteins from group A strains, and a homologous DNA sequence could not be detected by polymerase chain reaction or Southern blotting. In addition, Group A streptococci did not retain a large hyaluronan capsule like group C strains. These results indicated that the 56-kDa protein is an ectoprotein kinase specific for group C streptococci that regulates hyaluronan capsule shedding by phosphorylation.


Thus, the Lansing et al. reference cited by the Examiner does not, in fact, disclose the DNA or amino acid sequence of the hyaluronan synthase from *S. equisimilis* and thus, the present invention is novel over the Lansing et al. reference.

Thus, none of the most pertinent prior art references known to the inventors or cited in co-pending U.S. and foreign applications, teach, disclose or even suggest a hyaluronan synthase gene as recited in the currently amended claims.

In the event any outstanding issues remain that foreclose the Examiner's ability to **expediently** pass the currently amended claims

to issuance, the Examiner is urged to contact the Applicant's undersigned attorney to resolve any outstanding issues via telephonic conference.

Respectfully submitted,



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